Development of LAG3^{+} cell lines and their use for studying the LAG3 mechanism

Colin G Graydon[1], Shifa Mohideen[1], Keith R Fowke[1,2,3,4]

[1]Department of Medical Microbiology and Infectious Diseases, University of Manitoba
[2]Department of Community Health Sciences, University of Manitoba
[3]Department of Medical Microbiology, University of Nairobi
[4]Partners for Health and Development in Africa

Questions? Comments? Contact Colin at graydonc@myumanitoba.ca

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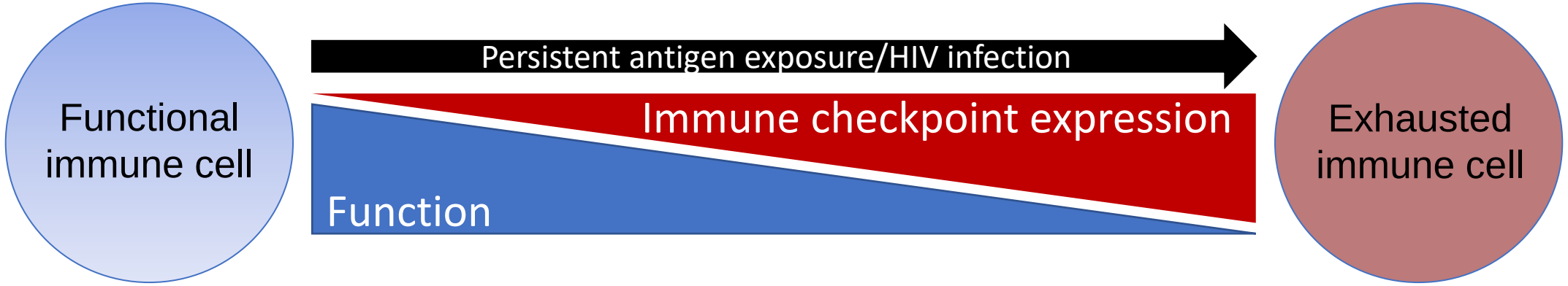
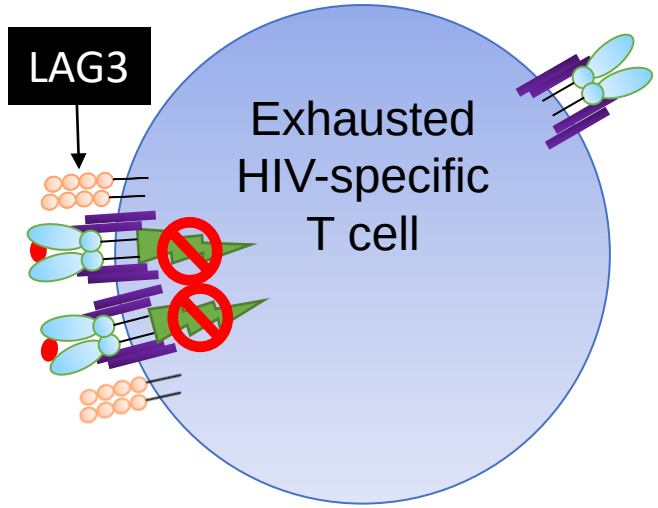


The immune checkpoint LAG3…

… is upregulated during persistent immune activation[1]

… reduces activation of immune cells (negative co-receptor)

… is a target for cancer immunotherapy, and a potential target for HIV functional cure and autoimmune therapies[5]



However, the mechanisms of LAG3 are unknown

- How does LAG3 inhibit T cell activation?
- How does LAG3 translocate to the cell surface?

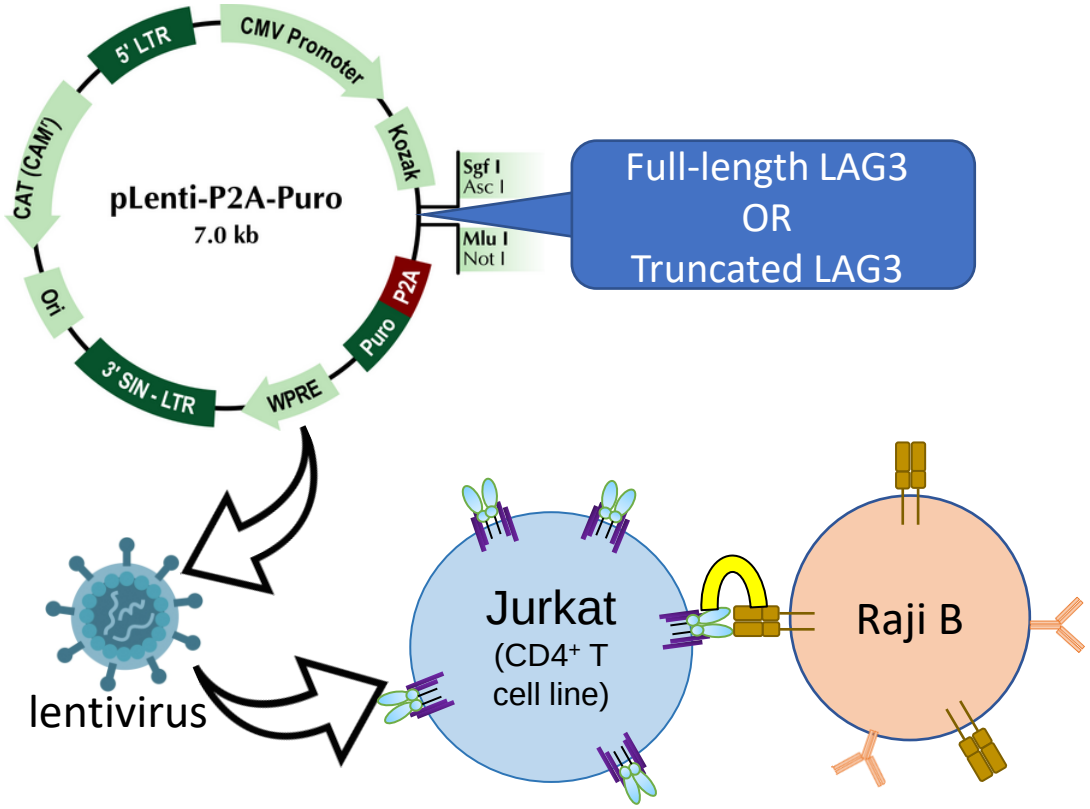
Objective:

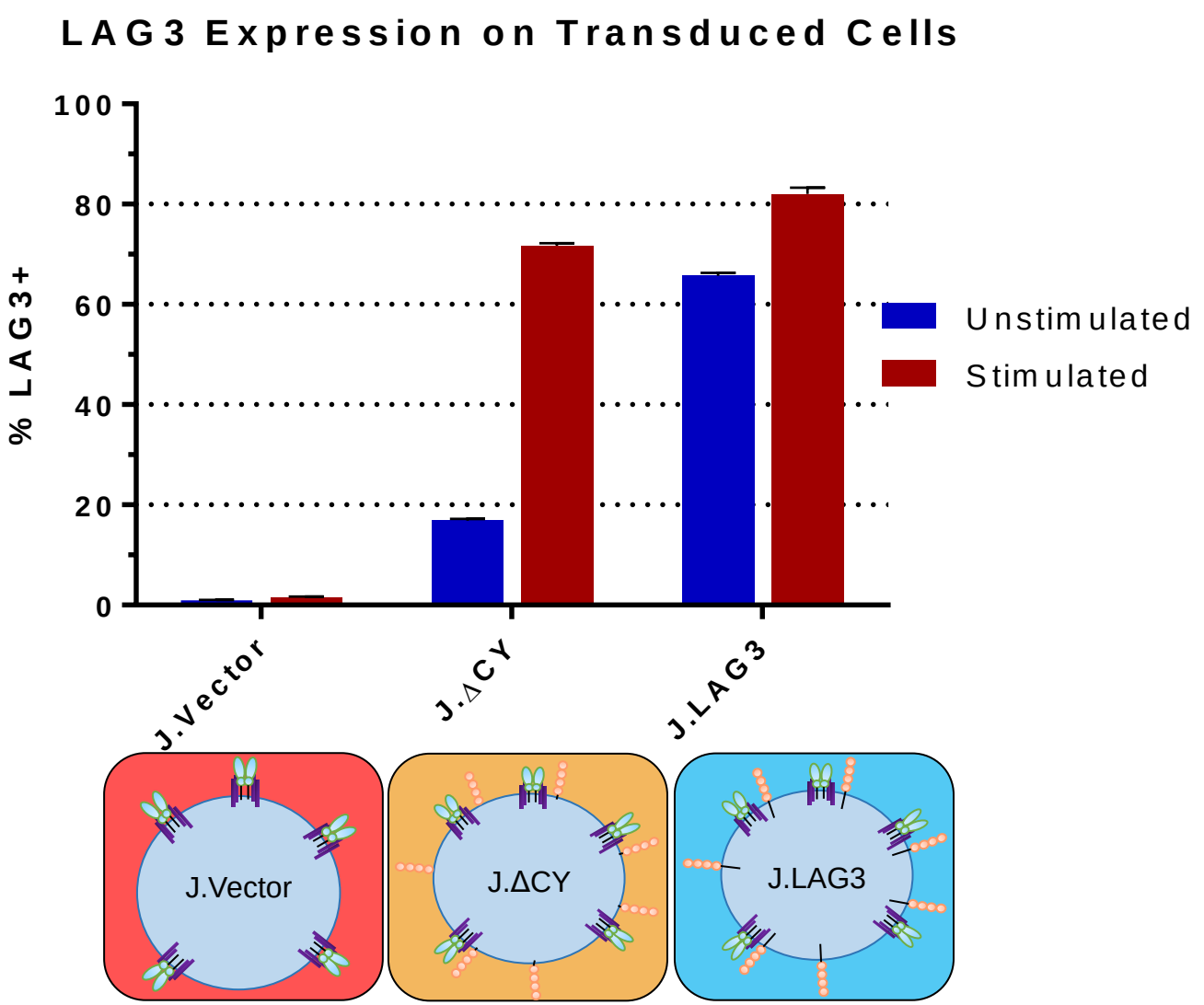
Create a model of LAG3 inhibition to help study the LAG3 mechanism of action

Methods:

Plasmid containing full-length LAG3 or LAG3 with the cytoplasmic domain deleted was packaged in lentivirus, then used to transduce the Jurkat cell line.

Transduced cells were activated with superantigen (SEE/SED) loaded Raji B cells





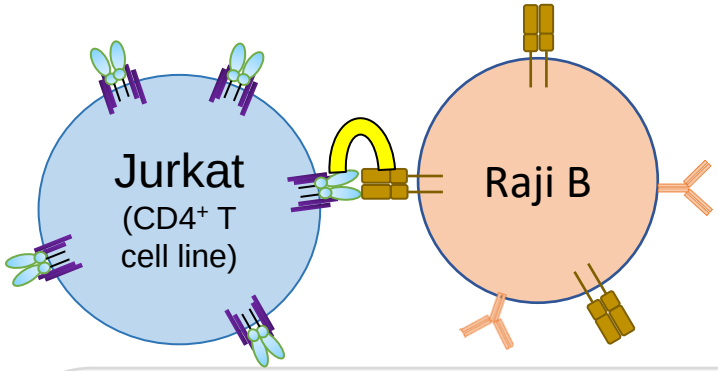
Conclusion:

1. Lentivirus transduction successfully overexpresses LAG3 in Jurkat cells.

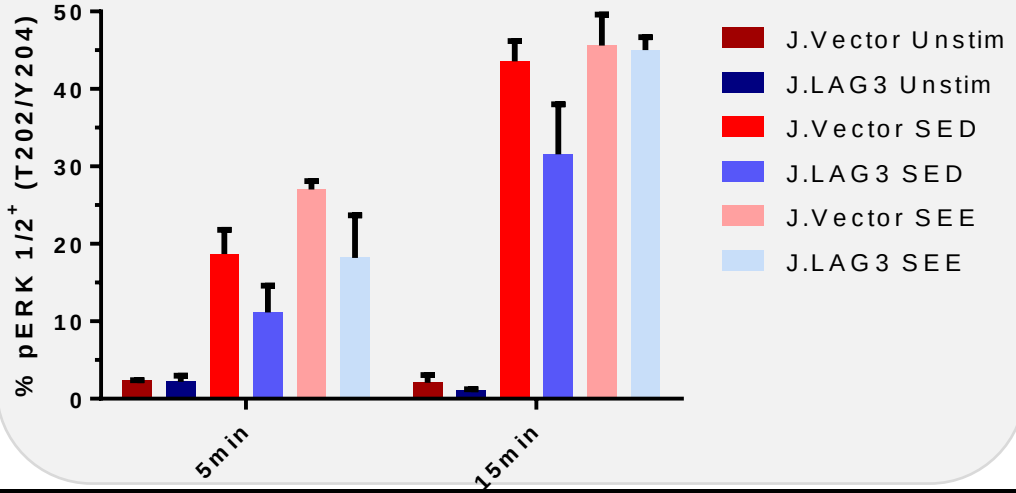
2. LAG3 lacking the cytoplasmic domain is not well expressed at the surface unless cells are activated.

Methods:

Transduced cells were activated by superantigen (SEE/SED) loaded Raji B cells. IL-2 was measured by ELISA and pERK ½ was measured by flow cytometry



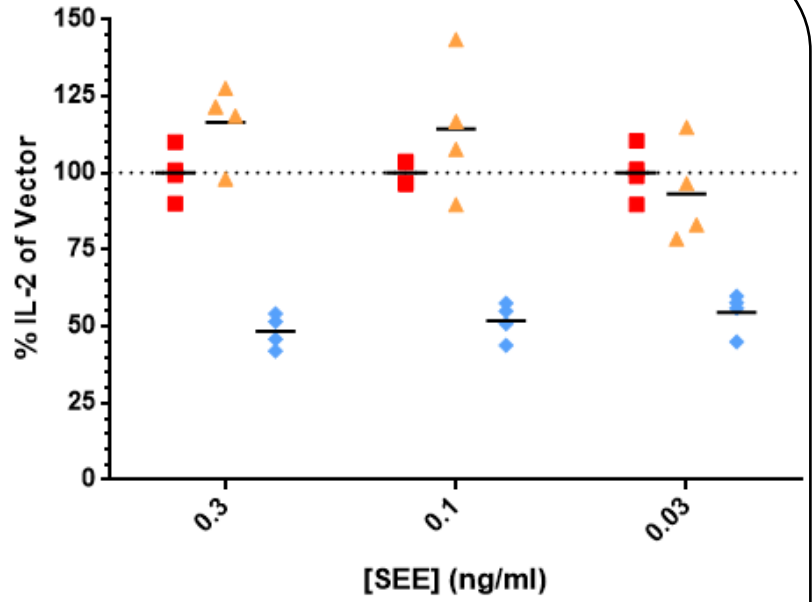
LAG3 inhibits pERK ½ (T202/Y204)

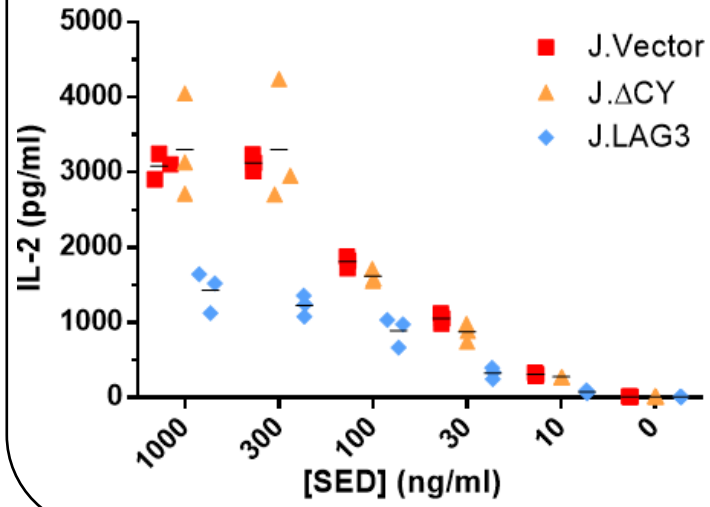


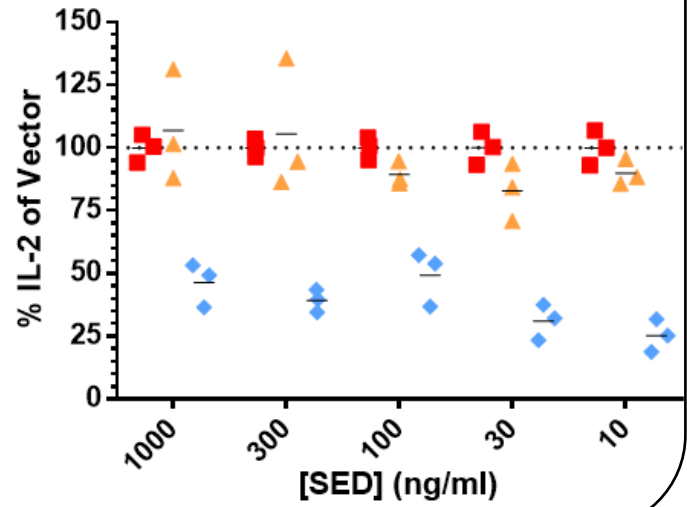
Conclusion:

3. LAG3 reduces early stage ERK ½ phosphorylation during superantigen stimulation

LAG3 inhibits IL-2 production







Conclusion:

4. LAG3 reduces IL-2 production by about half following stimulation with superantigen
5. This model effectively represents LAG3 inhibitory activity.

Conclusions

1. The LAG3 cytoplasmic domain is essential for full LAG3 expression in the absence of T cell activation
2. LAG3 inhibits IL-2 production and ERK phosphorylation in Jurkats
3. This model of LAG3 effectively replicates LAG3's inhibitory activities

Significance

- LAG3 inhibition could play a role in a functional cure by reversing latency and enhancing T cell immunity.
- The LAG3 mechanism of action is unknown largely because of a lack *in vitro* models for LAG3.
- This model could help derive the LAG3 mechanism of action, which would have wide implications for LAG3 inhibition.

References

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